Appendix 2.5 A

Task 2.5 A: Investigation of Ultraviolet Light Disinfection

Submitted by:

Alexander A. Mofidi, Bradley M. Coffey,
James F. Green, Connie I. Chou

Metropolitan Water District of Southern California
La Verne, California

Submitted to:

California Energy Commission
Sacramento, California

January 2002

LEGAL NOTICE

This report was prepared as a result of work sponsored by the California Energy Commission (Commission). It does not necessarily represent the views of the Commission, its employees, or the State of California. The Commission, the State of California, its employees, contractors, and subcontractors make no warranty, express or implied, and assume no legal liability for the information in this report; nor does any party represent that the use of this information will not infringe upon privately owned rights. This report has not been approved or disapproved by the Commission nor has the Commission passed upon the accuracy or adequacy of this information in this report.

ACKNOWLEDGEMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

The Public Interest Energy Research (PIER) Program supports public interest energy research and development that will help improve the quality of life in California by bringing environmentally safe, affordable, and reliable energy services and products to the marketplace.

The PIER Program, managed by the California Energy Commission (Commission), annually awards up to $62 million to conduct the most promising public interest energy research by partnering with Research, Development, and Demonstration (RD&D) organizations, including individuals, businesses, utilities, and public or private research institutions.

PIER funding efforts are focused on the following six RD&D program areas:

- Buildings End-Use Energy Efficiency
- Industrial/Agricultural/Water End-Use Energy efficiency
- Renewable Energy
- Environmentally-Preferred Advanced Generation
- Energy-Related Environmental Research
- Strategic Energy Research

What follows is the final report for *Electrotechnology Applications for Potable Water Production and Protection of the Environment,* Contract No. 500-97-044**,** conducted by the Metropolitan Water District of Southern California. The report is entitled "Electrotechnology Applications for Potable Water Production and Protection of the Environment: Task 5 Investigation of Ultraviolet Light Disinfection." This project contributes to the Industrial/Agricultural/Water End-Use Energy Efficiency area.

For more information on the PIER Program, please visit the Commission's Web site at: http://www.energy.ca.gov/research/index/html or contact the Commission's Publications Unit at 916-654-5200.

EXECUTIVE SUMMARY

Water utilities are constantly challenged with the problem of increasingly stringent disinfection regulations with disinfection byproduct (DBP) formation and would greatly benefit from a process that effectively inactivates recalcitrant organisms without increasing DBP risks. The objective of this study was to evaluate UV light from low-pressure, medium-pressure, and pulsed-UV lamps as a post-filtration disinfectant of bacteria, viruses, and protozoa.

UV light was demonstrated to be an effective disinfectant. A UV dose of 20 mJ/cm^2 was equivalent to 2.4 mg/L of chlorine for one minute or 2.4 mg/L of chloramines for one hour. The three treatment techniques each provided more than 3.5 log$_{10}$ (99.97 percent) inactivation of bacteria. UV disinfection alone was investigated for its ability to provide disinfection of *B. subtilis* aerobic spores, MS-2 coliphage (a single-stranded RNA virus), and phi-6 bacteriophage (a double-stranded RNA virus), organisms which may be used as surrogates when studying the disinfection of human pathogens. UV was effective in disinfecting these three organisms, with a dose of 40 mJ/cm^2 providing 1.9, 1.5, and 2.0 log$_{10}$ inactivation, respectively. UV was also investigated for its ability to inactivate *G. lamblia* cysts in water (results quantified with an animal infectivity assay), and study results found that a low UV dose of 1.4 mJ/cm^2 would provide a 2 log$_{10}$ inactivation of *G. lamblia*. UV disinfection experiments were conducted with another protozoan parasite, *C. parvum*, to determine if the low UV dosages used in disinfection may allow these organisms to repair themselves and become re-infective (at dosages up to 17 mJ/cm^2). However, within the amount of variability inherent in the *C. parvum* experiments conducted, no repair was observed.

Experiments compared the disinfection achieved by the medium-pressure and pulsed-UV lamps. The experiments were conducted to determine if the pulsed-UV lamp could enhance disinfection because of the higher irradiance during pulses. The experiments compared the inactivation of heterotrophic bacteria, *B. subtilis*, MS-2, phi-6, and *C. parvum*. Across all the experiments, there was no significant difference in the results obtained when using one lamp or the other for disinfection. This result was likely

because the effect of UV light on organisms probably results from the absorption of UV photons, and is not dependent on photon density.

UV alone, compared to UV followed by chloramines was evaluated to minimize bacterial regrowth and/or repair after treatment. When samples were treated with UV alone at dosages up to 60 mJ/cm^2, bacteria could nearly return to the initial density within three days. However, when UV was followed by a chloramine dose of 2.6 mg/L, bacteria did not regrow over seven days. Results indicate UV light to control post-filtration heterotrophic bacteria would necessitates application of a residual disinfectant (i.e., chloramines) to provide biological stability. UV treatment of filtered drinking water followed by chloramination produces fewer DBPs compared with chlorination (Mofidi et al. 1998).

Table 1 summarizes the UV disinfection results for the organisms evaluated in this study. Protozoa and heterotrophic bacteria were most susceptible to UV light, with dosages less than 20 mJ/cm^2 providing 2 log$_{10}$ (99 percent) inactivation. Organisms more resistant to UV light were the double-stranded RNA virus phi-6, *B. subtilis* and the single-stranded RNA virus MS-2. For these organisms, a UV dose of 40 – 53 mJ/cm^2 was required to provide 2 log$_{10}$ inactivation. UV was more effective against the human pathogen *G. lamblia* than previously reported for *G. muris* (Craik et al. 2000), a more easily handled rodent parasite. For future studies, *G. muris* may provide a conservative estimate of the disinfection of *G. lamblia* without the complexity the human pathogen or conducting the more difficult gerbil-based infectivity assay.

This study demonstrated that equivalent disinfection of each organism tested could be achieved no matter what lamp type was evaluated. Figure 1 demonstrates that both the medium-pressure and pulsed-UV lamps provided similar disinfection when compared on an equivalent UV dose measurement basis.

Table 1. Amount of UV dose required to provide 2-$\log_{10}$ inactivation of target organism

	ORGANISM TYPE	UV DOSE
		(mJ/cm^2)
	Giardia lamblia	<2
	Cryptosporidium parvum	<12
	Heterotrophic Bacteria	<20
	Phi-6 bacteriophage	40
	Bacillus subtilis	42
	MS-2 coliphage	53
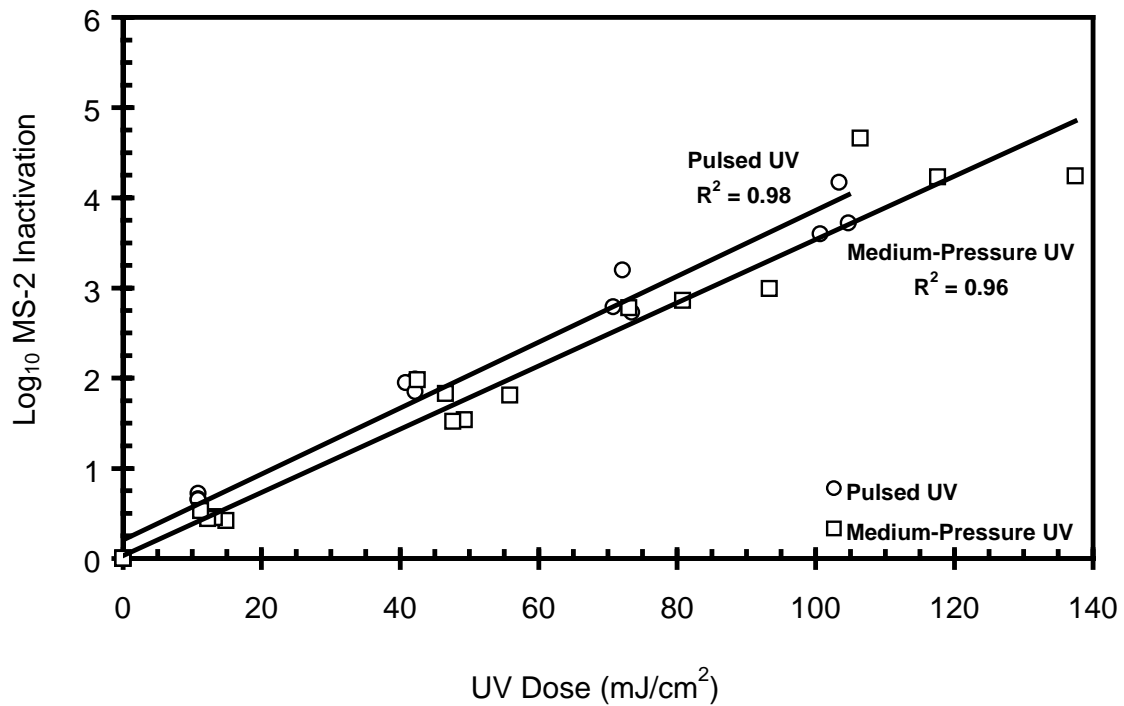
Figure 1. Effect of medium-pressure and pulsed-UV light on MS-2 coliphage

Microorganisms such as bacteria and protozoa maintain some ability to self-repair DNA damage by UV light. For example, *E. coli* can regain its ability to multiply following UV treatment (Harris et al. 1987). The studies conducted with heterotrophic bacteria, however, primarily showed that UV treatment could not prevent regrowth of the bacteria (using bacterial nutrients in the water).

Because *Cryptosporidium* requires an animal host to propagate, it would not regrow in treated water. However, the complexity and sophistication of the organism may provide some ability to repair non-lethal damage to DNA after some period of time. Within the precision of the *Cryptosporidium* infectivity assay, no repair was observed. These results should be revisited when the *Cryptosporidium* method improves.

The bench-scale research showed that UV light (from low-pressure, medium-pressure, and pulsed-UV) can effectively inactivate bacteria, protozoa, and viruses suspended in a filtered drinking water. Although UV disinfection systems are sold by a number of manufacturers, its use is relatively new in drinking waters. Currently, UV is only accepted as a technology to disinfect viruses. Pending additional research showing the effectiveness of UV against protozoa, UV may be accepted as a disinfectant against protozoa.

UV disinfection is fast becoming a great benefit to California water treatment utilities. However, the recommendations stated above should be followed before implementing large-scale UV technology. Although the process shows to be viable at the bench-scale, large-scale technology needs for on-line monitoring are still in development and should be evaluated before implementing the technology as a reliable barrier to waterborne diseases.

ABSTRACT

Ultraviolet light is receiving increased attention in the drinking water industry as a method for utilities to meet more stringent disinfection requirements while minimizing disinfection byproducts. However, there are unknowns regarding the required UV dosages for microorganism disinfection. This project studied (1) the UV dosages (compared to chlorine dosages) needed to control heterotrophic bacteria, (2) the needed UV dosages to disinfect aerobic spores, two different RNA viruses, and *Giardia lamblia*, and (3) the repair potential of UV-irradiated *Cryptosporidium parvum*. Many aspects of the research (bacteria, spore, and virus disinfection) were conducted to determine if an innovative pulsed-UV lamp technology could enhance disinfection compared to a traditional continuous-wave, medium-pressure lamp. Heterotrophic bacteria was seen to be easily disinfected (greater than 3-$\log_{10}$ reduction) by both pulsed and medium-pressure UV lamps at a dose of 20 mJ/cm^2. A similar disinfection level was observed with one minute of chlorine contact or one hour of chloramine contact. However, UV treatment could not prevent bacteria from regrowing to pre-treatment levels after 3 days. There was no regrowth seen after UV treatment of 20 mJ/cm^2 followed by 2.6 mg/L chloramines for up to 7 days. UV light from pulsed and medium-pressure lamps appeared to provide similar results when disinfecting *B. subtilis*, MS-2 coliphage, and phi-6 bacteriophage ($\geq$1.5 $\log_{10}$ disinfection of these organisms was achieved at 40 mJ/cm^2). Experiments with *G. lamblia* showed that dosages <2 mJ/cm^2 provided >2-$\log_{10}$ disinfection. Also, tests were inconclusive in determining whether or not repair mechanisms were present in the protozoa *C. parvum* up to 7 days after UV disinfection. Tests showed that less than 12 mJ/cm^2 of UV provided >2-$\log_{10}$ disinfection of *C. parvum*.

Keywords: bacteria, chlorine, chloramines, *Cryptosporidium*, disinfection, *Giardia*, UV, virus

INTRODUCTION

Background and Overview

Many California utilities (including Metropolitan Water District of Southern California) are implementing the energy-intensive ozonation process in order to meet more stringent chlorinated disinfection byproduct (DBP) regulations set by the United States Environmental Protection Agency (USEPA). In addition, biological filtration will also be implemented to remove readily biodegradable dissolved organic material produced by the ozonation process (Coffey et al. 1997). A secondary disinfectant is necessary downstream of biological filtration such that high levels of heterotrophic bacteria do not enter the distribution system (or regrow in the distribution system), compromising drinking water quality (e.g., interfering with coliform monitoring and colonizing the distribution system).

To compensate for the high levels of bacteria after biological filtration, Metropolitan will employ a short period of free-chlorine contact to reduce heterotrophic bacteria to less than 10 colony forming units per milliliter (CFU/mL). After this, ammonia will be added to form chloramines, a weaker disinfectant that persists for long contact times in the distribution system and minimizes the formation of chlorinated DBPs. According to a study conducted by Metropolitan (Yates 1998), the resulting level of chlorinated DBPs formed through this process (after treating a blend of California surface waters) are expected to be in the range of 30 microgram per liter (µg/L) trihalomethanes (THMs) and 15 µg/L haloacetic acids (HAAs). The current regulated levels of THMs and HAAs are 80 and 60 µg/L, respectively (USEPA 1998). From previous research, Mofidi and co-workers (1998) have found that biological filtration followed by pulsed-UV irradiation and chloramines treatment could achieve HPC levels of <10 CFU/mL with THM and HAA levels of <10 µg/L each. However, the UV and chloramine doses to prevent bacterial regrowth in the distribution system have not been established. It is necessary to understand these dosages and compare them with the dosages of chlorine and chloramines which provide comparable biological filter-effluent bacterial disinfection.

UV light affects both regrowth and repair of heterotrophic bacteria. Hengesbach and co-workers (1993) have shown that UV irradiation does not enhance the growth of heterotrophic bacteria when compared to unirradiated waters. However, Stewart and co-workers (1993) have shown that regrowth of bacteria does occur after UV treatment if a secondary disinfectant is not applied. Numerous investigators have also seen that *Escherichia coli*, a coliform bacteria which does not have regrowth potential in aqueous environments, does have the ability to repair itself depending on the magnitude of delivered UV dose (Kelner 1951; Lindenauer and Darby 1994; Tosa and Hirata 1999).

UV lamps are now shown to provide excellent disinfection of protozoa (Clancy et al. 1998; Bukhari et al. 1999; Mofidi et al. 2000). This study evaluated the potential of medium-pressure and pulsed-UV lamps to disinfect aerobic spore forming bacteria (*Bacillus subtilis*) and the two virus surrogates MS-2 coliphage (single stranded RNA structure) and phi-6 bacteriophage (double stranded RNA structure). These organisms were chosen to determine if they could be used as easier-to-handle and less-expensive surrogates for *C. parvum*. Also, it is unknown if phi-6 may prove to be even more resistant to UV than MS-2. If this is the case, phi-6 may be applicable as a surrogate for recalcitrant pathogenic viruses such as Reovirus (double-stranded RNA virus).

Because low doses of UV light have been shown to inactivate *C. parvum* oocysts (Mofidi et al. 2000), the ability for *C. parvum* to repair itself after exposure to these low doses and become infectious again is an important consideration. The basis for conducting this work relates to reports in the literature where irradiated DNA-based bacteria have shown potential for repair after UV-induced disinfection (Harris et al. 1987, Kelner 1951, Mechsner et al. 1991; Lindenauer and Darby 1994, Tosa and Hirata 1999). If the DNA of irradiated *C. parvum* shows that it can repair itself after low doses of UV light, the actual UV doses needed to sustain irreparable damage to *C. parvum* in drinking water may be higher than anticipated. This increase in the required UV dose could possibly increase the power requirements for UV disinfection of *C. parvum*.

Giardia lamblia, another protozoan, causes as many as 2.5 million cases of giardiasis in the United States alone each year, approximately 25 percent of which are waterborne in nature (Furness 2000). In drinking water, *G. lamblia* is typically controlled with both physical removal and disinfection of cysts. Only recently has it been shown that UV light is an effective disinfectant against *G. lamblia* (Craik et al. 2000). This research showed that viability assays (i.e., excystation) do not adequately quantify UV light effects on cysts, necessitating the use of animal infectivity assays. To understand if UV affects *G. lamblia* similarly to *C. parvum*, this research investigated the infectivity of *G. lamblia* in Mongolian gerbils after exposure to low dosages of UV light.

Project Objectives

The objectives of this project task were as follows:
- Evaluate the ability of heterotrophic bacteria to repair and/or regrow following UV treatment;
- Compare disinfection effectiveness of pulsed UV and medium-pressure UV lamps against the single-stranded RNA virus MS-2 (which is a surrogate for human enteric viruses and polio);
- Evaluate the disinfection effectiveness of UV lamps against two organisms which may be a disinfection surrogate for *Cryptosporidium*, phi-6 (a double-stranded RNA virus) and Bacillus subtilis (a bacteria encapsulated in a spore structure with double-stranded DNA);
- Determine the ability of *Cryptosporidium parvum* to self-repair its infectivity after exposure to UV light; and,
- Determine the disinfection effectiveness of UV light against Giardia lamblia, another protozoan pathogen found in drinking water.

PROJECT APPROACH

The disinfection experiments for this project were conducted with low-pressure (for very low UV dosages required in *G. lamblia* tests), medium-pressure, and pulsed-UV lamps at the bench-scale as shown in Figure 1 and Figure 2. Microorganisms exposed to UV light

from the low- and medium-pressure UV lamps were done so with a collimated-beam unit (Calgon Carbon Corp., Pittsburgh, Penn.) illustrated in Figure 1. The lamps are mounted 3 in. above 2.5-in. inside-diameter polyvinyl chloride (PVC) collimating tube with the interior of the tube painted flat black to minimize reflected light. A pneumatic shutter had opening/closing times of less than 0.5 sec. Collimated experiments exposed continuously stirred suspensions of microorganisms in 0.5-cm (0.2-in.) deep, 10-mL volumes contained in a 60-mm (2.4-in.), sterile, Permanox tissue-culture dish (Nalge Nunc International, Rochester, N.Y.). Exposure times were adjusted to keep the shutter interference time at less than five percent of the total exposure time. The collimating tube could length was either 10 in., 20 in., or 50 in. UV irradiance was measured by radiometer (model IL 1700, SED240 detector with W diffuser; International Light, Inc., Newburyport, Mass.) and a potassium-iodide actinometer described elsewhere (Rahn 1997, Rahn et al. 2000). The dose of germicidal UV light transferred to the microorganisms was calculated after measuring the UV light beam's center irradiance value before and after exposing each sample. This center value was then pro-rated for locations away from the center according to an irradiance distribution determined at the beginning of the study. To determine this variation in intensity across the water surface, UV irradiance was measured once at the beginning of the study across a 0.5-cm (0.2-in.) planar grid at 25 locations. Averaged radiometer readings were adjusted by absorbance of the natural water matrix according to the governing principles of the Beer-Lambert Law as described by Morowitz (1950). UV irradiance was then multiplied by the exposure time to determine a dose of germicidal UV light, measured in mJ/cm^2. UV dose for the pulsed-UV experiments was measured by the potassium-iodide actinometer. A correlation between radiometer-measured dose and actinometer-measured dose was made so that results from the low- and medium-pressure UV lamps could be compared to the pulsed-UV lamp.

Inactivation and Regrowth of Heterotrophic Bacteria

A standard repair/regrowth protocol calls for samples to be irradiated and placed in a controlled environment for a specific time. This study called for the potential of heterotrophic bacteria to re-grow and repair after UV irradiation to be evaluated in both a

lighted (i.e., simulated open-air storage reservoir) and dark (i.e., simulated pipeline distribution system) environment. Water samples exposed to UV light were taken from a 6 gallons-per-minute (gpm) pilot plant operating in the ozone/biofiltration mode described elsewhere (Mofidi et al. 1998). Aliquots of irradiated samples were placed in sterile petri dishes in the following controlled laboratory environments. To determine the contribution of bacterial repair compared to regrowth potential, aliquots of heterotrophes were inoculated into filter-sterilized natural water (filtered to 0.22 µm) and incubated to establish baseline heterotrophic regrowth potential. A daylight environment was simulated by exposure to two 15 W daylight simulator lamps (Vitalite; Duro-Test Corp., Fairfield, N.J.) suspended above the samples as shown in Figure 3. These lamps provided non-ionizing visible radiation to simulate water storage in an open-air reservoir after treatment. Samples were also incubated in petri dishes in a dark cabinet to simulate the distribution system. The temperature of both samples remained at ambient conditions.

Experiments were conducted in the following test matrix:
- Heterotrophic bacteria inactivation after contact with chlorine and chloramines;
- Heterotrophic bacteria inactivation after exposure to pulsed-UV light;
- Heterotrophic bacteria inactivation after exposure to pulsed-UV light and addition of chloramines (up to 7 days storage in a simulated distribution system);
- Proliferation of heterotrophic bacteria after exposure to pulsed-UV light and placement in both a simulated open-air reservoir and simulated distribution system (up to 7 days storage); and,
- Baseline heterotrophic regrowth potential in bacteria-inoculated, filter-sterilized water placed in both simulated environments (up to 7 days storage).
- Incubation environments were temperature controlled (to 20 ^{o}C) and aliquots of sample were taken from the petri dishes at 3 and 7 days (72 and 168 hours).

Test procedures were conducted in a controlled and repeatable manner for each test. First, water was collected for the UV experiments at the beginning of each day. Next, the pulsed-UV system's UV dosage characteristics were measured before each test by using

the procedures outlined above. The chlorine/chloramine experiments were conducted by adding chlorine either alone, at the same time, or at a staggered time with ammonia solution into the test volume of water. If required, chloramines were added following UV treatment. Time-zero heterotrophic bacteria samples were analyzed to understand the immediate bacteria response to treatment. Controls, UV exposed samples, and UV/chloramine samples were then placed in the appropriate light and dark environments for incubation. After 1 day, the first set of aliquots were taken from the incubating samples and analyzed. Subsequently, after 7 days of incubation, an aliquot from the sample remaining in the petri dish was again taken for analysis.

Inactivation of *Bacillus subtilis* Spores, MS-2 Coliphage, and Phi-6 Bacteriophage

Experiments evaluated several UV doses from 10 to 100 mJ/cm^2 for disinfection of the different microorganisms. Organisms were suspended in the high-quality (high UV transmission, low particulate matter) pre-treated water described for the bacteria experiments. Disinfection procedures (i.e., UV dose measurement and UV exposure protocol) with the UV units were similar to that described previously for heterotrophic bacteria.

As with heterotrophic bacteria, test procedures were conducted in a controlled and repeatable manner for each experiment. First, water was collected for the UV experiments at the beginning of each day. Next, the UV systems' dosage characteristics were measured by using the dose measurement procedures described above (both the medium-pressure and pulsed-UV lamps were used in this testing). The process water collected for the experiment was then spiked with organisms (only one organism type per experiment), exposed to UV light, and sent to the laboratory for immediate analysis.

Inactivation of *Giardia* and *Cryptosporidium*

Giardia Lamblia

Each *Giardia* test consisted of six samples: four samples exposed to different dosages of UV light (low-pressure UV lamp used because a very low UV irradiance was required) and two experiment controls (one positive and one negative). The positive control and the four samples exposed to step-dosages of UV light contained statistically similar amounts of suspended *Giardia*. Across the tests, the level of *G. lamblia* ranged from 0.96×10^5 to 1.9×10^5 cysts/mL, respectively. Negative controls consisted of filtered water without adding *Giardia*.

After each test, 4 dilutions were made of each sample and typically 3 gerbil hosts were fed 0.2 mL of each sample dilution. On occasion 4 gerbils were used to increase statistical precision. Other research recently presented determined loss of *G. muris* cyst infectivity after UV exposure due to a shift in the latent period of cyst shedding (Craik et al. 2000). Work presented here measured the inactivation of *Giardia* in the samples exposed to UV light by quantifying the number of animals that became infected with *Giardia* per dilution (i.e., positive results). This effect provided a 3-host code. For example, if serial dilutions of 0.02, 0.002, 0.0002, and 0.00002 mL resulted in 3,3,2, and 1 animals infected, *Standard Methods* (APHA 1998) describes the resulting 3-host code to be 3-2-1. This code, combined with the dilution information, was then interpreted by most probable number (MPN) calculation and an MPN value was assigned the outcome (see Materials and Methods section, below). The difference between MPN values for each experiment's positive control and the UV-exposed samples resulted in the calculated inactivation of *Giardia* cysts.

Cryptosporidium Parvum

Tests examined the disinfection efficiency (and DNA repair potential) of *C. parvum* after at least three doses of UV radiation from both a pulsed-UV and a medium-pressure UV

lamp (a low, medium, and high dose). Experiments were conducted in the following matrix:

- Irradiation of *C. parvum* with two UV technologies;

- Irradiation of *C. parvum* with three UV doses; and,

- Quantification of *C. parvum* reduction immediately after UV exposure and after a 1-week incubation.

All experiments were joined by an appropriate number of control samples to (1) compensate for any possible effects that pulsed UV simmer has on experiment results and (2) appropriately characterize organism response with several positive and negative controls. The procedure for the irradiation experiments are identical for the organisms previously mentioned. *C. parvum* samples examined for DNA repair, as stated above, were assayed one week post-irradiation to quantify changes in oocyst infectivity compared with oocysts examined immediately after irradiation (the same day). Naturally shed oocysts of *C. parvum* were used in the infectivity/repair studies (University of Arizona, Department of Veterinary Science, Tucson, Ariz.).

Materials and Methods

Alkalinity

Alkalinity was determined by titration, as described in *Standard Methods* (APHA 1998).

Bacillus Subtilis

B. subtilis (American Type Culture Collection [ATCC] strain 6633; ATCC, Rockville, M.D.) were grown on trypan blue agar plates for 2 to 3 days at 37 ^{o}C and then incubated at room temperature for 3 to 4 days to form spores from nutrient exhaustion. Organisms were removed from plates, re-suspended in sterile phosphate-buffered water, and heated at 82 ^{o}C for 15 minutes, and then immediately stored at 4 ^{o}C until ready for UV exposure. To kill vegetative cells and induce spores to germinate, samples were placed in an 82 ^{o}C

water bath until samples reached 82 $^{\circ}$C. After reaching this target temperature, samples remained in the bath for 15 additional minutes and then were stored at 4 $^{\circ}$C. At this point, samples were exposed to UV light. Post-exposure, and after vigorous shaking, samples were filtered through 0.45-µm filters which were then incubated at 37 $^{\circ}$C for 24 hr, and spore growth on the filter paper was then counted. Results are presented as cfu/mL. Serial dilutions of the samples were processed and only dilutions which indicate 20 to 200 colonies (on each plate) were used in quantitation. Every dilution was run in triplicate plates.

Cryptosporidium Parvum

C. parvum oocysts were obtained from the University of Arizona, from calves artificially infected with the *C. parvum* genotype 2 Iowa isolate originally obtained from Dr. Harley Moon, of the National Animal Disease Center (Ames, Iowa). Once harvested from the infected calves, the oocysts were cleaned by sequential centrifugation through sucrose and cesium chloride and placed in an antibiotic solution (100 µg/mL penicillin, 100 µg/mL streptomycin, 100 µg/mL gentamicin) containing 0.01 percent polyoxyethylenesorbitan monolaurate ("Tween 20") to prevent clumping. The oocysts were then shipped to Metropolitan, where they were stored at 4°C and used within two weeks. The oocysts were further purified by immunomagnetic separation with Dynabeads[®] (Dynal, Inc., Lake Success, N.Y.) and then counted by hemocytometer (Bright-line; Hausser Scientific, Horsham, Pa.). Excystation rates were determined by a modification of the method described by Robertson and Campbell (1993). Oocysts were used for experiments only if the exhibited excystation rates were >90 percent. Five small squares were counted on both sides of the hemocytometer (10 squares total), and the average count was calculated. All oocyst stocks had been previously counted by the University of Arizona before shipment. If Metropolitan's counts deviated from these initial counts by more than ten percent, the hemocytometer count was repeated. On average, the relative standard deviation was less than 20 percent among the 10 square counts obtained.

Suspensions of *C. parvum* oocysts were prepared by spiking a known number of oocysts of *C. parvum* into pretreated, oxidant-free, natural water collected from the pilot plant (the same water used in the bacteria and virus tests). UV-irradiated samples containing *C. parvum* were transferred into 15-mL polypropylene centrifuge tubes and transferred on ice to the laboratory, where they were processed immediately—with (1) a concentration step (centrifugation at $1050 \times g$ for 15 min at 19°C [66°F]) to produce a 1-mL sample and (2) a 5- or 10-fold serial dilution of the pellet in pilot plant water (for both samples and positive controls)—and then inoculated onto the cell monolayers.

The infectivity of *C. parvum* was determined by cell culture, using a modification of the method that is described elsewhere (Rochelle et al. 1997, Mofidi et al. 2000) and briefly here. A confluent monolayer of human illeocecal carcinoma cells (HCT-8, ATCC CCL 244; American Type Culture Collection, Rockville, Md.) was grown in single-well slides (Lab-Tek; Nalge Nunc International, Rochester, N.Y.) over 2–4 days, infected with oocysts of *C. parvum*, and incubated at 37°C for 48 h in 5-percent carbon dioxide. Total ribonucleic acid (RNA) was extracted from the cell monolayers with S.N.A.P kits (Invitrogen Corp., Carlsbad, Calif.), and the messenger RNA fraction was purified using oligo (dT) cellulose (Micro-FastTrack kit; Invitrogen Corp.). *C. parvum*-specific heat-shock protein (*hsp*) 70 gene transcripts were detected by RT-PCR, using primers previously described by Rochelle et al. (1997). The presence of infectious oocysts in samples produced the specific 361-base pair (bp) amplicons, whereas uninfected cells or inactivated oocysts did not produce amplicons. Following gel electrophoresis, PCR products were confirmed by hybridization with a *C. parvum*-specific oligonucleotide probe.

Following PCR amplification and gel electrophoresis, gel images were digitized using a flatbed scanner (Epson ES-1200C; Sieko Epson Corp., Nagano-ken, Japan), and band intensities were quantified by densitometry using commercially available software (Pro-Quanta©; DNA ProScan, Inc., Nashville, Tenn.). Calibration curves were constructed by plotting $\log_{10}$ concentrations of oocysts (obtained from dilutions of the *C. parvum* stock solution used) against the signal intensity. This curve was then used to determine the

number of infectious oocysts based on the signal intensity of each unknown experimental sample.

The sample dilution and calibration curve approach described above was developed to overcome the nonquantitative limitations of PCR amplification. A MPN technique was investigated. Diluted samples were assayed by cell culture with the goal of obtaining a breakpoint where no infection would be detected. However, experiments indicated that the band intensities on the electrophoresis gels were proportional to the concentration of infectious oocysts under the specific PCR conditions employed. Either 25 or 30 cycles were conducted with the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primer and 35 or 40 cycles with the *C. parvum*-specific primers. Under these conditions, the band intensities of the unknown samples were compared with the band intensities of the calibration curve made up with a known number of infectious oocysts. It was then possible, without MPN, to determine the number of infectious oocysts associated with each unknown sample.

Each experiment set included three negative controls: (1) a noninfected slide; (2) heat-inactivated (70°C for 10 min); and (3) pilot-plant effluent water (presumably oocyst free). RT and PCR negative controls, consisting of sterile molecular-grade water in the RT or PCR mixes, were processed along with the experimental samples. If a negative control showed a signal, the experiment was not considered valid.

Positive controls were also processed during each experiment. Amplification of a 600-bp amplicon from the human GAPDH gene (Stratagene, La Jolla, Calif.) after RNA extraction provided an RT-PCR positive control for every amplification reaction. An additional PCR positive control consisted of previously extracted DNA of *C. parvum*. A serial dilution of nonirradiated oocysts of *C. parvum* was also inoculated onto a series of cell monolayers to generate a calibration curve. The experimental positive controls consisted of aliquots of suspended oocysts of *C. parvum* contained in either the quartz cuvette (for pulsed-UV lamp experiments) or the petri dish (for medium-pressure experiments), but not exposed to UV radiation. In the case of the pulsed-UV lamp, an

11

additional positive control was added that consisted of a cuvette containing an aliquot of a suspension of oocysts of *C. parvum* exposed to the lamp's simmer mode. This control was added to evaluate the oocyst inactivation resulting from possible UV light emitted by the partial ionization of the lamp.

Giardia Lamblia

Giardia cysts (WB strain) were obtained from Dr. Merle Olson at the University of Calgary as trophozoites in culture. This line was isolated 15 years ago from a human patient who became infected in Afghanistan and has been widely circulated to many laboratories. It has since been maintained by serial passage in culture and by cryopreservation. Cultured WB trophozoites were inoculated into gerbils acquired from the University of Calgary. The WB strain cysts for these experiments were passed three times through gerbils before use. Gerbils were provided with food and water as needed and dexamethasone (30 mg/mL) was added to their drinking water to enhance cyst production.

G. lamblia cysts were isolated from gerbil feces by homogenization, screening and discontinuous flotation on 1.0 M sucrose using the underlay method at the Hyperion Research laboratory (Medicine Hat, Alberta, Canada). Centrifugation was at 1,000*g* for 6 min. Cysts trapped at the interface were disrupted with a stir stick, transferred to a fresh 50 mL conical centrifuge tube and again concentrated by centrifugation. Cysts were washed three times by resuspending in deionized water and centrifuging. The suspension was aged for 24 hours at 4 °C and then refloated and rewashed to reduce background turbidity. The final suspension was quantified by counting in a Neubauer hemocytometer. One drop of Tween-20 was added to the preparation to minimize clumping. The semi-purified suspension was shipped to Metropolitan's Water Quality laboratory overnight (in accordance with government pathogen transportation guidelines) in a glass vial with neoprene stopper crimped with metal foil, surrounded by packing material and ice.

Upon 24-hr arrival at Metropolitan, shipments where inspected to ensure that *G. lamblia* cyst suspensions remained cold and did not freeze during shipment. Cysts were counted by hemocytometer and suspensions of equivalent number of cysts were made with the pilot plant water. Table 1 shows the range of the filtered water quality throughout the project. The filtered water was a blend of California State project water and Colorado river water pre-treated at one of Metropolitan's treatment plants by ozone pre-oxidation followed by biological filtration. The cyst suspensions were exposed to various levels of UV light (according to the disinfection procedures outlined below) and shipped overnight to Oregon Health Sciences University (OHSU, Portland, Ore.) for subsequent inoculation into the appropriate animal hosts.

Within 24-hr arrival at OHSU, *G. lamblia* cyst suspensions previously exposed to UV light at Metropolitan were inoculated into female gerbils (provided with food and water as needed). Gerbils were maintained in cages with air-filter tops to prevent cross-contamination. Ten-fold dilutions of each cyst suspension were made and a 0.2 mL volume of each dilution was inoculated into the gerbil esophagus with a 26 gauge needle attached to a syringe. Samples were concentrated 10-fold before inoculation into the gerbils resulting in the highest possible dilution of sample being 2.0 ml fed to the gerbils in a 0.2 mL volume. Ten days post inoculation, feces were collected from individual gerbils and examined microscopically for cysts. Peak cyst excretion was found by other researchers on days thirteen (Visvesvara et al. 1988) and fifteen (Belosevic et al. 1983) therefore feces were also collected from individual gerbils and examined microscopically for the presence of cysts fourteen days post-inoculation. Gerbils producing cyst-negative feces on day fourteen were euthanized by overdose of carbon dioxide and their small intestines examined microscopically for *Giardia* trophozoites. This was accomplished by dissecting out the duodenum and jejunum, mixing scrapings from the inner surface in a drop of water on a slide, and performing a microscopic examination. Gerbils were considered to be infected by *G. lamblia* when either trophozoites were found in the small intestines or cysts were found in the feces. For the gerbils found positive during these tests, 65 percent were by fecal presence of cysts and 35 percent were by intestinal presence of trophozoites.

13

Hardness

Hardness (total) was measured with EDTA titration to define the sum of calcium and magnesium concentrations, and expressed as calcium carbonate (mg/L) as described in *Standard Methods* (APHA 1998).

Heterotrophic Bacteria

Heterotrophic bacteria were analyzed using membrane filtration and incubation on R2A media at 28 $^\circ$C for 7 days, according to *Standard Methods* (APHA 1998). Plating was conducted in triplicate, and results are presented as cfu/mL.

Most Probable Number Determination

The Most Probable Number (MPN) technique was first introduced in order to estimate bacterial densities from fermentation tube test results (McCrady 1915) and uses statistical analysis to provide a modal value of organism density based on probability theory (Thomas 1942). Since its introduction, the literature describes the MPN method as providing normally distributed results (Eisenhart 1943) which can be characterized by standard deviation and 95 percent confidence limits (Woodward 1957). Due to the mathematical complexities of the MPN technique, it was difficult to apply to a dilution series that did not conform to pre-published tables (Hoskins 1933). Upon the advent of modern personal computers, MPN equations which produce data in published tables have been automated to accurately estimate organism density, and reliance on published tabular values is not necessary. MPN equations developed by Hurley and Roscoe (1983) have been incorporated into readily available MPN calculation software (MPN calculator, version VB6; Mike Curiale, Silliker laboratories, Homewood, Ill). This Hurley and Roscoe-based MPN calculator was used to calculate MPN values for the dilution series used in this study.

MS-2 Coliphage

MS-2 coliphage was obtained from American Type Culture Collection (ATCC strain 15597-B1; ATCC, Rockville, M.D.), with *Escherichia coli* Famp (ATCC strain 15597) as the bacterial host. The assay was conducted according to the procedure described in the Information Collection Rule (USEPA 1996a, USEPA 1996b), along with an added purification stage, and is recommended by the USEPA for simulating the inactivation of enteric viruses (USEPA 1982). MS-2 was grown onto tryptone agar plates, and resuspended into a saline-calcium buffer solution. MS-2 stock solution was purified by centrifugation (15 minutes at 10,000xg and 4 °C) followed by membrane filtration (0.22 μm porosity). The added purification, conducted prior to each experiment, further purified an aliquot of MS-2 stock solution with spin columns (TE Super Select-D, G-50; 5'→3', Inc., Boulder, Colorado) to remove any contaminants that may interfere with UV irradiation. The resulting titer was diluted to approximately 10^8 plaque forming units per milliliter (PFU/mL).

E. coli Famp was incubated in tryptone broth overnight at 37 °C, then transferred into new tryptone broth and incubated for 4 hours at 37 °C to allow maximum pili expression. Propagation was performed using a double-agar overlay procedure (Adams 1959) described by Wolfe et al. (1989). One mL of the *E. coli* 4-hour culture was added to test tubes containing warm (45 °C) tryptone agar, immediately followed by the addition of 1 mL of MS-2 sample for appropriate dilution. The test tube was rapidly rolled between the palms of the analyst's hands and dispensed over a tryptone agar petri plate. Plates were incubated for 20 hours at 37 °C. Samples were run in triplicate.

Positive controls consisted of MS-2 coliphage stock solution before and after spin column purification. Negative controls consisted of sterile saline-calcium solution (experiment negative control), *E. coli* not infected with MS-2, and sterile media to account for agar contamination in the incubator.

pH

Water pH was analyzed by a pH meter with Accuracy of ±0.02 pH units (model 920A; Orion Research, Inc., Boston, Mass.)

Phi-6 Bacteriophage

Phi-6 bacteriophage (*Pseudomonas syringae pathovar phaseolicola* bacteriophage phi-6, ATCC 21781-B1) was assayed similarly to MS-2 coliphage (a plaque assay). The host for phi-6 is the plant pathogen *Pseudomonas syringae pathovar phaseolicola* (ATCC 21781). The host was grown in nutrient broth yeast extract (NBY) media and optimum plaquing was observed when the host was grown for two consecutive 24 hr periods at 28 ^{o}C with agitation at 120 rpm prior to infecting with phi-6 phage. Plaque assay was performed by the top agar method in NBY media supplemented with 1 percent agar and incubated overnight at room temperature.

Temperature

Temperature was measured using a thermometer calibrated against a certified thermometer by the National Bureau of Standards.

Turbidity

Turbidity was measured with a Hach 2100N Turbidimeter (Hach Company, Loveland, Colo.) calibrated with dilute formazin solutions as specified by the manufacturer with an accuracy of ±2 percent.

UV Light Absorbance at 254 nm

UV light absorbance at 254 nm in a 1-cm quartz cuvette was measured by a spectrophotometer (Lambda 3B, Perkin Elmer Corp., Wellesley, Mass.), and is reported in units of cm^{-1}.

PROJECT OUTCOMES

Inactivation and Regrowth of Heterotrophic Bacteria

Results of the chlorine/chloramine tests are summarized in Figure 4. Data points represent median density of heterotrophic bacteria remaining after a dose of 2.6 ± 0.1 (average $\pm$ standard deviation) mg/L of chlorine and chloramines were applied for different contact times. Vertical bars drawn through the median data represent minimum and maximum counts of bacteria for the tests. Data show that a 61-minute contact with chloramines ($4.3 \log_{10}$ inactivation) provided similar disinfection when compared to a brief 1-minute contact with chlorine ($3.9 \log_{10}$ inactivation) or a 1-minute contact with Cl_2 followed by a 60 minute contact with chloramines ($4.1 \log_{10}$ inactivation). A 1-minute contact with chloramines only provided $2.2 \log_{10}$ inactivation of bacteria.

Levels of heterotrophic bacteria in the filtered water (before treatment by UV or chlorine) were initially 10,000 CFU/mL. The inoculated regrowth control—consisting of filter sterilized water spiked with a low concentration of heterotrophic bacteria—had an initial bacterial density of <10 CFU/mL. Exposure to 20 or 60 mJ/cm^2 of UV light reduced the heterotrophic bacteria to ≤ 10 CFU/mL. Test results were similar when either the medium-pressure or pulsed-UV lamp was evaluated.

Tests conducted to investigate the disinfection of heterotrophic bacteria with UV light from medium-pressure and pulsed-UV lamps applied dosages of 20 and 60 mJ/cm^2 for both lamps. A dose of 20 mJ/cm^2 inactivated the bacteria by 3.7 ± 0.2 and $3.3 \pm 0.5 \log_{10}$ from the medium-pressure and pulsed-UV lamp, respectively. At the dose of 60 mJ/cm^2, inactivation of bacteria increased slightly to 3.9 ± 0.3 and $3.8 \pm 0.7 \log_{10}$. Previously reported data indicate more than 3-$\log_{10}$ inactivation of heterotrophic bacteria can be achieved with UV dosages less than 20 mJ/cm^2 (Mofidi et al. 2000). The data at 20 and 60 mJ/cm^2 described above may indicate an effect of tailing in the disinfection effectiveness of UV light on the mixed bacterial populations.

Data from the disinfection experiments which studied repair and regrowth characteristics of heterotrophic bacteria are shown in Figure 5 and Figure 6. Data from tests with the two different lamps appear similar within the variability of the results. Variability in results is attributed to the both the multiplication of heterotrophic bacteria during the experiment and the assay technique used to quantify the number of bacteria present on different days. Each experiment was completed with a filter effluent control not exposed to UV light, a regrowth control, two samples exposed to UV light (20 and 60 mJ/cm^2), and a third sample exposed to 20 mJ/cm^2 and subsequently treated with 2.5 mg/L of chloramines.

Samples placed in a dark incubation environment (simulating a distribution system environment) were tested for presence of heterotrophic bacteria three and seven days later, as shown in Figure 5. Treatment by 20 or 60 mJ/cm^2 of UV light reduced the heterotrophic bacterial density by greater than 3 $\log_{10}$. The viable bacteria following UV treatment typically recovered to the original density. A regrowth control—described above—also recovered to the original density. The regrowth control test confirmed that conditions of the sample such as temperature, nutrients, and holding duration were sufficient for bacterial growth. As such, the repair of bacteria following UV light exposure was insignificant compared to normal bacterial growth.

The presence of chloramines following UV treatment was sufficient to prevent continued bacterial growth. Figure 5 shows that samples treated by UV and maintained by a chloramine dose of 2.6 mg/L could control bacteria to less than 3 CFU/mL.

Results from tests treating samples and incubated them in the simulated daylight environment (mimicking post-treatment discharge to an open-air reservoir or lake) are presented in Figure 6. Three replicate tests (only one test shown) showed similar results. One test had a UV/chloramines sample, and showed only 1 CFU/mL of bacteria after the 7-day incubation. Both UV-only treated samples, the filter effluent sample, and the regrowth sample contained >65,000 CFU/mL after 7 days.

Results from both the daylight-simulated and dark tests indicate that bacterial repair is insignificant to heterotrophic bacterial growth in samples which contain sufficient nutrients, are at a high enough temperature, or are held for enough days.

Inactivation of Bacillus Subtilis Spores, MS-2 Coliphage, and Phi-6 Bacteriophage

Tests investigated the susceptibility of *B. subtilis*, MS-2, and phi-6 to UV light from both a medium-pressure and pulsed-UV lamp. The data collected during these tests produced dose-response relationships for all three microorganisms, as shown in Figure 7, Figure **8**, and Figure **9**.

Figure 7 shows a relationship between UV dose from both lamps and inactivation of *B. subtilis* spores. The data sets from the medium-pressure and pulsed-UV lamps show strong correlation between UV dose and inactivation of *B. subtilis* (R^2 values of 0.92 and 0.96, respectively) and indicate that the effect of UV light from both lamps show similar response of inactivation of *B. subtilis*. At a dose of 40 mJ/cm^2, the linear regressions indicate experimentally-similar results: medium-pressure UV would provide 1.8 log$_{10}$ inactivation and pulsed UV would provide 2.1 log$_{10}$ inactivation.

Figure 8 summarizes the relationship seen between UV dose from the two lamps and its effect on MS-2 coliphage. Similar to the *B. subtilis* results, the data sets from both lamps show strong correlation between UV dose and inactivation of MS-2 ($R^2 = 0.96$ for medium-pressure and $R^2 = 0.98$ for pulsed UV). Also, the data indicate that the effect of UV light from either lamp shows a similar response as measured by inactivation of MS-2. Calculating the inactivation of MS-2 achieved at 40 mJ/cm^2 by using the linear regression of the data sets, medium-pressure UV provides 1.4 log$_{10}$ while pulsed UV provides 1.7 log$_{10}$. This is seen to be similar within the constraints of the tests conducted, but lower than other tests which show more than 2-log$_{10}$ reduction at 40 mJ/cm^2 (Wilson et al. 1992).

Lastly, Figure 9 shows the relationship seen between UV dose from the two lamps and the inactivation of phi-6 bacteriophage. These test results confirm the results

19

characterized by the tests with both *B. subtilis* and MS-2 whereas dose-response was seen to be similar between the samples exposed to medium-pressure and pulsed-UV light. At a dose of 40 mJ/cm^2, medium-pressure provided 1.9 log$_{10}$ inactivation of phi-6 while pulsed UV provided 2.1 log$_{10}$ inactivation.

None of these organisms were as susceptible to UV light as *C. parvum*. Mofidi and co-workers (2000) showed that *C. parvum* was reduced by 2 log$_{10}$ at a dose of 6.5 mJ/cm^2.

If the medium-pressure and pulsed-UV data for the each of the three organisms studied were to be combined (as shown in Figure 10), there would be a high correlation of the linear regression for each set. Under these conditions, *B. subtilis* data has an R^2 = 0.94 and the regression shows 1.9 log$_{10}$ inactivation at a dose of 40 mJ/cm^2. MS-2 and phi-6 have R^2 values of 0.96 and 0.92, respectively, and a dose of 40 mJ/cm^2 provides 1.5 log$_{10}$ and 2.0 log$_{10}$ inactivation, respectively. Above 50 mJ/cm^2, disinfection results becamed more variable.

Inactivation of *Giardia* and *Cryptosporidium*

Tests investigated the susceptibility of *G. lamblia* and *C. parvum* to UV light. For *G. lamblia*, tests were conducted with a low-pressure UV lamp so that very low UV dosages could be attained. For the *C. parvum* tests, both the medium-pressure and a pulsed-UV lamps were used. Data collected during these tests produced a dose-response relationship for *G. lamblia*. However, the purpose of the *C. parvum* tests was not to establish a dose-response relationship, but to identify if the organism could repair itself after treatment (that is, show less disinfection following storage than immediately after UV exposure).

UV Dose-Giardia Lamblia Cyst Response

Over a period of six months there were five different *G. lamblia* tests conducted. Figure **11** illustrates a compilation of the results from all tests. *G. lamblia* was readily disinfected by UV light. According to a linear regression of the data (R^2 = 0.63), a dose

of 1.2 mJ/cm^2 could provide a 2-log$_{10}$ inactivation of *G. lamblia*. Thus, *G. lamblia* is less resistant to UV than both *C. parvum* and the bacterium *Escherichia coli* (Mofidi et al. 2000, Harris et al. 1987, Schoenen 1996). *G. lamblia* may also be less resistant to UV light than the easier to handle rodent parasite *G. muris* (Craik et al. 2000).

Cryptosporidium Parvum Repair

Initial disinfection results for the *C. parvum* experiments are presented in Figure 12 while the data illustrating change in inactivation across the 7-day incubation are presented in Figure 13. Figure 12 demonstrates that results for the disinfection portion of the *C. parvum* experiments were highly variable. At dosages ranging from 4.1 to 4.5 mJ/cm^2, the response of the inactivation of *C. parvum* oocysts was seen to range from 0.6 to 2.8 log$_{10}$ for pulsed UV and from 1.9 to >5.2 log$_{10}$ for medium-pressure UV. This variability was seen at all the dosages studied, which ranged from 4 to 18 mJ/cm^2. The combined data from both lamps produced a low dose-response correlation with an $R^2 = 0.40$. The most significant difficulty experienced during the study was in obtaining data which did not exceed the limits of the infectivity assay. This is evident when reviewing oocyst response from UV dosages between 8 to 16 mJ/cm^2 (for either lamp technology) where *C. parvum* data is indicated as beyond the detection limit (i.e., the actual reported inactivation is unknown, but is at least equal to or greater than what is reported by the filled data points). However, within the variability which was experienced, disinfection results from the more traditional medium-pressure UV lamp were not shown to be significantly different than the disinfection achieved by the pulsed-UV lamp. This result is similar to what was found by Mofidi and co-workers (2000).

Due to the significant number of data points which were beyond the detection limit of the infectivity assay, very few experiments could be analyzed to determine repair potential of *C. parvum* oocysts. As shown in Figure 13, there are 8 data points (3 from medium-pressure experiments and 5 from pulsed-UV experiments) available to characterize the change in inactivation across the 7-day post-disinfection incubation period. After exposure to UV light, incubation of the UV-treated oocysts was conducted at 20 ^{o}C (experiment controls were placed in storage for the same period of time at 4 ^{o}C). The

repair potential of *C. parvum* oocysts was determined by comparing the infectivity of oocysts after initial exposure to UV light (data presented in Figure 12) to the infectivity of oocysts which were exposed to UV and placed in the incubation for 7 days. If the infectivity of oocysts in the UV treated samples increased after 7 days (when compared to the stock sample of non-exposed oocysts), then it was concluded that storage of oocysts resulted in enhancing the disinfection effects of UV light. However, a decrease in the change in oocyst infectivity after 7 days could indicate that repair mechanisms may be present. Data are available from three different UV doses ranging from 4 to 17 mJ/cm^2. The data at 4 mJ/cm^2 show mixed results, with 3 data points (one from a medium-pressure and two from a pulsed-UV test) indicating increases in inactivation after 7 days (a range from 0.2 to 2.4 log$_{10}$). At 12 mJ/cm^2, one medium-pressure UV data point shows an increase in inactivation of 0.1 log$_{10}$. However, at 4 mJ/cm^2, there were two data points (one medium-pressure and one pulsed-UV) showing decreases in inactivation from 0.2 to 0.7 log$_{10}$, and at 17 mJ/cm^2 there were two data points indicating decreases of 0.9 log$_{10}$ (pulsed UV result) and 1.6 log$_{10}$ (medium-pressure result).

Due to the variability in the infectivity assay, test results are inconclusive. The data show both increases (increased inactivation) and decreases in inactivation (possible oocyst repair) across the range of UV dosages investigated without any clear trends in the data. Also, at the highest UV dosages (17 mJ/cm^2) where theory may suggest that possible repair mechanisms may be overcome by the increased UV dose, both samples from both lamps indicated decreases in inactivation. These data need to be seen in light of the very high variability of the disinfection results presented in Figure 12.

Summary

The ability of UV light to disinfect bacteria, viruses, and protozoa suspended in a filtered drinking water was evaluated in this study. The immediate effects of 20 mJ/cm^2 of UV light against heterotrophic bacteria was seen to be equivalent to the more traditional chemical disinfectants chlorine (1 minute contact) and chloramines (61 minutes contact). The three treatment techniques each provided more than 3.5 log$_{10}$ inactivation of bacteria. UV disinfection alone was investigated for its ability to provide disinfection of *B. subtilis*

aerobic spores, MS-2 coliphage (a single-stranded RNA virus surrogate), and phi-6 bacteriophage (a double-stranded RNA virus surrogate). UV also seen to be effective in disinfecting these three organisms, with a dose of 40 mJ/cm^2 providing 1.9, 1.5, and 2.0 log$_{10}$ inactivation, respectively. UV alone was also investigated for its ability to provide disinfection of *G. lamblia* cysts in water (results quantified with an animal infectivity assay), and study results found that a very low UV dose of 1.4 mJ/cm^2 would provide a 2 log$_{10}$ inactivation of *G. lamblia*. UV disinfection experiments were conducted with another protozoan parasite, *C. parvum*, to determine if the low UV dosages used in disinfection may allow these organisms to repair themselves and become re-infective (at dosages up to 17 mJ/cm^2). However, within the amount of variability inherent in the *C. parvum* experiments conducted, it could not be concluded whether or not repair mechanisms exist which can overcome UV disinfection.

Experiments also evaluated the disinfection achieved by a conventional medium-pressure UV lamp compared to that from an innovative pulsed-UV lamp. The experiments were conducted to determine if the innovative lamp design could enhance the disinfection achieved compared with the more conventional lamp type. The experiments investigated the two lamps' ability to inactivate heterotrophic bacteria, *B. subtilis*, MS-2, phi-6, and *C. parvum*. Across all the experiments, there appears to be no significant difference in the results obtained when using one lamp or the other for disinfection.

This study also evaluated the effects of UV only, compared with the effects of UV followed by addition of chloramines, on the biological stability of treated samples (characterized by the regrowth and/or repair of heterotrophic bacteria after treatment). When samples were treated with UV dosages up to 60 mJ/cm^2, the amount of bacteria typically was reduced to levels of less than 10 CFU/mL. However, after incubation to simulate effects which a distribution system may have on post-irradiated water, it was seen that levels of bacteria could re-establish pre-treatment levels within a 3-day period. However, if samples were treated by a UV dose of 20 mJ/cm^2 and followed with a chloramine dose of 2.6 mg/L, the samples could remain biologically stable for at least

7 days. Samples treated by UV/chloramines kept heterotrophic bacteria levels below 5 CFU/mL.

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The effects that UV disinfection has on various organisms, as presented in this study, is summarized in Table 2. The most susceptible organisms to UV light were found to be protozoa and heterotrophic bacteria, with UV dosages of less than 20 mJ/cm^2 providing 2 log$_{10}$ inactivation. Organisms more resistant to UV light were the double-stranded RNA virus phi-6, followed by *B. subtilis* and then the single stranded RNA virus MS-2. For these organisms, a UV dose between 40 and 53 mJ/cm^2 was required to provide 2 log$_{10}$ inactivation. The disinfection provided by UV on the human pathogen *G. lamblia* was even more effective than what has been previously reported for *G. muris* (Craik et al. 2000), a more easily handled rodent parasite.

This study shows that the process of using UV light to control post-filtration heterotrophic bacteria would need to be followed by a residual disinfectant such as chlorine or chloramines to provide a water with biological stability. Previous studies (Mofidi et al. 1998) have demonstrated that UV treatment of a filtered drinking water followed by chloramination would also provide a water which is also low in chlorinated disinfection byproducts.

This study demonstrated that similar disinfection of many different types of organisms could be achieved by either a more traditional medium-pressure, continuous-wave UV lamp or an innovative pulsed-UV lamp type. The disinfection provided by both lamps was similar when compared on an equivalent UV dose basis. Both lamps were effective in the treatment of *C. parvum*, but it could not be determined whether or not *C. parvum* could repair itself following UV treatment. Future studies should be conducted to determine if *C. parvum* repair mechanisms may exist after UV treatment. Because of the similar disinfection achieved with different UV lamp types, these future studies could be

limited to one lamp type (such as the low-pressure UV lamp used in the *G. lamblia* studies reported here).

Although some variability in the *C. parvum* dose-response relationship could be explained both by the nature of the infectivity assay and by the inherent variability of the oocysts, the variability seen here was excessive. To better quantify effects of organism repair in future studies, it would be beneficial to wait until improvements in *C. parvum* infectivity assays are made so that variability is reduced.

Commercialization Potential

The research conducted in this report was done at the bench-scale, but showed that UV light (from low-pressure, medium-pressure, and pulsed-UV) can effectively inactivate bacteria, protozoa, and viruses suspended in a filtered drinking water. The UV disinfection process is a technology currently made available by a number of manufacturers, although it is a new process for the treatment of drinking waters. Pending future research into the ability of the process to scale-up from the bench-top to large-scale UV reactors (treating several million gallons of water per day), the process needs additional research to determine its limitations before large-scale implementation.

Recommendations

North American utilities (such as the Metropolitan Water District of Southern California) that treat surface water understand the limitations inherent in monitoring disinfection performance within large-scale UV reactors. Future research must complement the bench-scale data presented here and elsewhere by evaluating the process efficiency and hydraulic characteristics of large-scale UV reactors. These evaluations should make recommendations for monitoring of transferred UV dosage and reporting of continuous disinfection effectiveness (i.e., on-line UV dosage measurement) so that drinking water treatment requirements can be met.

Benefits to California

UV disinfection is fast becoming a great benefit to California water treatment utilities. However, the recommendations stated above should be followed before implementing large-scale UV technology. Although the process shows to be viable at the bench-scale, large-scale technology needs for on-line monitoring are still in development and should be evaluated before implementing the technology as a reliable barrier to waterborne human disease and illness.

REFERENCES

Adams, M. H. (1959). Bacteriophages. New York, Interscience Publishers, Inc.

APHA (American Public Health Association), AWWA (American Water Works Association) and WEF (Water Environment Federation) (1998). Standard Methods for the Examination of Water and Wastewater. Washington, D.C., American Public Health Association.

Belosevic, M., G. M. Faubert, J. D. MacLean, C. Law and N. A. Croll (1983). "Giardia lamblia Infections in Mongolian Gerbils: An Animal Model." Journal of Infectious Diseases 147: 222-226.

Bukhari, Z., T. M. Hargy, J. R. Bolton, B. Dussert and J. L. Clancy (1999). "Medium-Pressure UV for Oocyst Inactivation." Journal American Water Works Association 91(3): 86-94.

Clancy, J. L., J. R. Bolton and B. Dussert (1998). Inactivation of Cryptosporidium parvum Oocysts Using Medium-Pressure Ultraviolet Light in Bench and Pilot Scale Studies. Water Quality Technology Conference, San Diego, CA, AWWA.

Clancy, J. L., Z. Bukhari, T. M. Hargy, J. R. Bolton, B. W. Dussert and M. M. Marshall (2000). "Using UV to Inactivate Cryptosporidium." Journal American Water Works Association 92(9): 97-104.

Coffey, B.M.; Huck, P.M.; Emelko, M.B.; Bouwer, E.J.; Hozalski, R.M.; Pett, B.; & Smith, E.F. (1997) The Effect of BOM and Temperature on Biological Filtration: An Integrated Comparison at Two Treatment Plants. AWWA Water Quality Technology Conference, Denver, Colo.

Craik, S. A., G. R. Finch, J. R. Bolton and M. Belosevic (2000). "Inactivation of Giardia muris Cysts Using Medium-Pressure Ultraviolet Irradiation in Filtered Drinking Water." Water Research 34(18): 4325-4332.

DuPont, H. L., C. L. Chappell, C. R. Sterling, P. C. Okhuysen, J. B. Rose and W. Jakubowski (1995). "Infectivity of Cryptosporidium parvum in Healthy Volunteers." The New England Journal of Medicine 332(13): 855-859.

Eisenhart, C. and P. W. Wilson (1943). Statistical Methods and Control in Bacteriology. Bacteriology Review. 7.

Finch, G. R., E. K. Black, C. W. Labatiuk, L. Gyurek and M. Belosevic (1993). "Comparison of Giardia lamblia and Giardia muris Cyst Inactivation by Ozone." Applied and Environmental Microbiology 59(11): 3674-3680.

Furness, B. W., M. J. Beach and J. M. Roberts (2000). Giardiasis Surveillance--United States, 1992-1997. Morbidity and Mortality Weekly Report. C. f. D. C. a. Prevention. Atlanta, Georgia, CDC. 49: 1-13.

Harris, G. D., V. D. Adams, D. L. Sorensen and M. S. Curtis (1987). "Ultraviolet Inactivation of Selected Bacteria and Viruses with Photoreactivation of the Bacteria." Water Research 21(6): 687-692.

Hengesbach, B., D. Schoenen, O. Hoyer, H. Bernhardt, G. Mark, H.-P. Schuchmann and C. von Sonntag (1993). "UV Disinfection of Drinking Water - The Question of Bacterial Regrowth and the Photolytic Degradation of Biogenic High-Molecular-Weight Substances." Journal Water Science Research and Technology-AQUA 42(1): 13-22.

Hoff, J. C., E. W. Rice and F. W. Schaefer III (1985). "Comparison of Animal Infectivity and Excystation as Measures of Giardia muris Cyst Inactivation by Chlorine." Applied and Environmental Microbiology 50(4): 1115-1117.

Hoskins, J. K. (1933). "The Most Probable Numbers of B. coli in Water Analysis." Journal American Water Works Association 25(6): 867-877.

Hurley, M. A. and M. E. Roscoe (1983). "Automated Statistical Analysis of Microbial Enumeration by Dilution Series." Journal of Applied Bacteriology 55: 159-164.

Karanis, P., W. A. Maier, H. M. Seitz and D. Schoenen (1992). "UV Sensitivity of Protozoan Parasites." Journal Water Science Research and Technology - AQUA 41(2): 95-100.

Kelner, A. (1951). "Action Spectra for Photoreactivation of Ultraviolet-Irradiated Escherichia Coli and Streptomyces Griseus." The Journal of General Physiology 34: 835-851.

Lindenauer, K. G. and J. L. Darby (1994). "Ultraviolet Disinfection of Wastewater: Effect of Dose on Subsequent Photoreactivation." Water Research 28(4): 805-817.

McCrady, M. H. (1915). "The Numerical Interpretation of Fermantation-Tube Results." Journal of Infectious Diseases 17: 183-212.

Mechsner, K., T. Fleischmann, C. A. Mason and G. Hamer (1991). "UV Disinfection: Short Term Inactivation and Revival." Water Science and Technology 24(2): 339-342.

Mofidi, A. A., H. Baribeau, J. F. Green, R. De Leon and R. L. Wolfe (1998). <u>Disinfection Using High-Intensity Pulsed-Ultraviolet Irradiation</u>. American Water Works Association Water Quality Technology Conference, San Diego, CA, AWWARF.

Mofidi, A. A., H. Baribeau, P. A. Rochelle, R. De Leon, B. M. Coffey and J. F. Green (submitted 2000, resubmitted 2001). "Disinfection of Cryptosporidium parvum with Polychromatic UV Light." <u>Journal American Water Works Association</u>.

Morowitz, H. J. (1950). "Absorption Effects in Volume Irradiation of Microorganisms." <u>Science</u> 111: 229.

Ortega, Y. R. and R. D. Adam (1997). "Giardia: Overview and Update." <u>Clinical Infectious Diseases</u> 25(3): 545-549.

Rahn, R. O. (1997). "Potassium Iodide as an Chemical Actinometer for 254 nm Radiation: Use of Iodate as an Electron Scavenger." <u>Photochemistry and Photobiology</u> 66(4): 450-455.

Rahn, R.O.; Bolton, J.R.; & Stefan, M.I. (2000) The Iodide/Iodate Actinometer in UV Disinfection: Characteristics and Use in the Determination of the Fluence Rate Distribution in UV Reactors. AWWA Water Quality Technology Conference, Salt Lake City, Utah.

Rendtorff, R. C. (1954). "The Experimental Transmission of Human Intestinal Protozoan Parasites." <u>American Journal of Hygiene</u> 59.

Rice, E. W. and J. C. Hoff (1981). "Inactivation of *Giardia* lamblia Cysts by Ultraviolet Irradiation." <u>Applied and Environmental Microbiology</u> 42(3): 546-547.

Robertson, L. J. and A. T. Campbell (1993). "In Vitro Excystation of Cryptosporidium parvum." <u>Parasitology</u> 106(13).

Rochelle, P. A. (2000). Detection and Occurrence of Giardia in the Environment. Encyclopedia of Environmental Microbiology. S. Dowd, John Wiley & Sons, Inc.

Rochelle, P. A., D. M. Ferguson, T. J. Handojo, R. DeLeon, M. H. Stewart and R. L. Wolfe (1997). "An Assay Combining Cell Culture with Reverse Transcriptase PCR to Detect and Determine the Infectivity of Waterborne *Cryptosporidium parvum*." Applied and Environmental Microbiology 63(5): 2029-2037.

Schaefer III, F. W., C. H. Johnson, C. H. Hsu and E. W. Rice (1991). "Determination of Giardia lamblia Cyst Infective Dose for the Mongolian Gerbil (Meriones unguiculatus)." Applied and Environmental Microbiology 57(8): 2408-2409.

Schoenen, D. (1996). "The Influence of Inhomogeneous Irradiation in UV Disinfection - Experimental Findings and Theoretical Considerations." J. WSRT-AQUA 45(3): 120-129.

Stewart, M. H., C. J. Hwang, P. A. Hacker, R. S. Yates and R. L. Wolfe (1993). Microbial and Chemical Implications of Using Ultraviolet Irradiation for Treatment of Biological Filter Effluent. 1993 American Water Works Association Water Quality Technology Conference, Miami, Florida, AWWA.

Thomas Jr., H. A. (1942). "Bacterial Densities From Fermentation Tube Tests." Journal American Water Works Association 34(4): 572-576.

Tosa, K. and T. Hirata (1999). "Photoreactivation of Enterohemorrhagic Escherichia Coli Following UV Disinfection." Water Research 33(2): 361-366.

USEPA (United States Environmental Protection Agency) (1982). Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources. Washington, D.C., Office of Drinking Water.

USEPA (1996a). ICR Microbiology Laboratory Manual. Washington, D.C., USEPA office of Research and Development.

USEPA (1996b). Monitoring Requirements for Public Drinking Water Supplies, Final Rule. Federal Register. Washington, D.C. 61:94:24354.

USEPA (1998). National Primary Drinking Water Regulations: Disinfectants and Disinfection Byproducts; Final Rule. Federal Register. Washington, D.C. 63:241:69390.

Visvesvara, G. S., J. W. Dickerson and G. R. Healy (1988). "Variable Infectivity of Human-Derived Giardia lamblia Cysts for Mongolian Gerbils (Meriones unguiculatus)." Journal of Clinical Microbiology 26(5): 837-841.

Wickramanayake, G. B., A. J. Rubin and O. S. Sproul (1984). "Inactivation of Giardia lamblia Cysts with Ozone." Applied and Environmental Microbiology 48(3): 671-672.

Wilson, B.R.; Roessler, P.F.; Van Dellen, E.; Abbaszadegan, M.; & Gerba, C.P. (1992) Coliphage MS-2 as a UV Water Disinfection Efficacy Test Surrogate for Bacterial and Viral Pathogens. 1992 American Water Works Association Water Quality Technology Conference, Toronto, Ontario, Canada, AWWA.

Wolfe, R. L., M. H. Stewart, K. N. Scott and M. J. McGuire (1989). "Inactivation of Giardia muris and Indicator Organisms Seeded in Surface Water Supplies by Ozone and PEROXONE." Environmental Science & Technology 23(6): 744-745.

Woodward, R. L. (1957). "How Probable is the Most Probable Number?" <u>Journal American Water Works Association</u> 49(8): 1060-1068.

Yates, R. (1998) Disinfection By-Product Formation Resulting from Ozonation and Short-Term Chlorination of Biological Filter Effluent. Metropolitan Water District of Southern California.

TABLES AND FIGURES

Table 1. Water quality of filtered water used to suspend cysts for disinfection experiments

Characteristic	25th Percentile Value	Median Value	75th Percentile Value
Hardness (mg/L as calcium carbonate)	183	207	211
Light absorbance at 254 nm (1/cm)	0.027	0.029	0.029
Light transmittance at 254 nm (percent)*	93.5	93.5	94.0
pH (units)	7.9	8.0	8.0
Turbidity (NTU)	0.07	0.08	0.09

* Calculated value based on the absorbance and using the Beer-Lambert law

Table 2. Amount of UV dose required to provide 2-$\log_{10}$ inactivation of target organism

Organism Type	UV Dose (mJ/cm^2)
Giardia lamblia	1.4
Cryptosporidium parvum	<12
Heterotrophic Bacteria	<20
Phi-6 bacteriophage	40
Bacillus subtilis	42
MS-2 coliphage	53
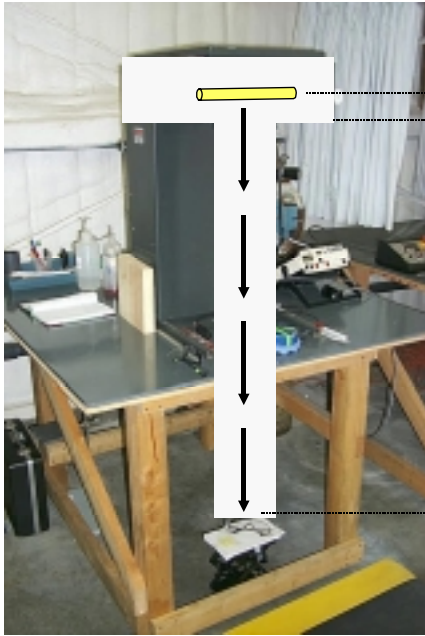
(a) Medium-Pressure Lamp Configuration   (b) Low-Pressure Lamp Configuration
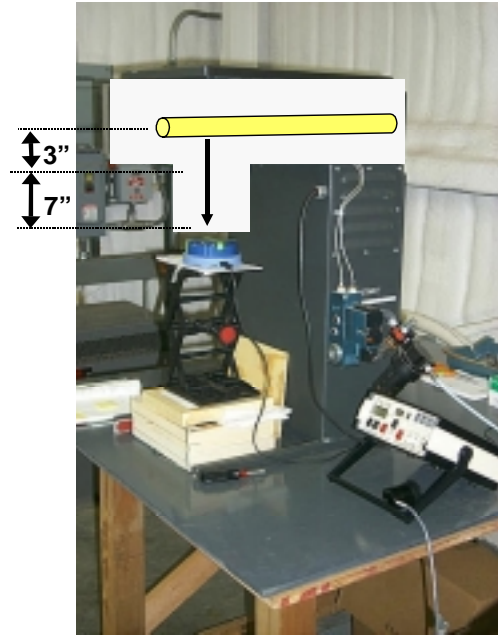
Figure 1. Continuous-wave collimated beam (two distances shown are the distance from the lamp to the collimating tube and the length of the collimating tube)
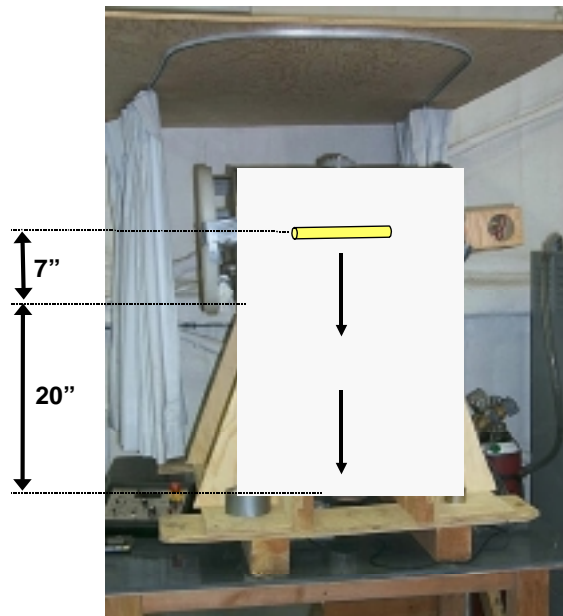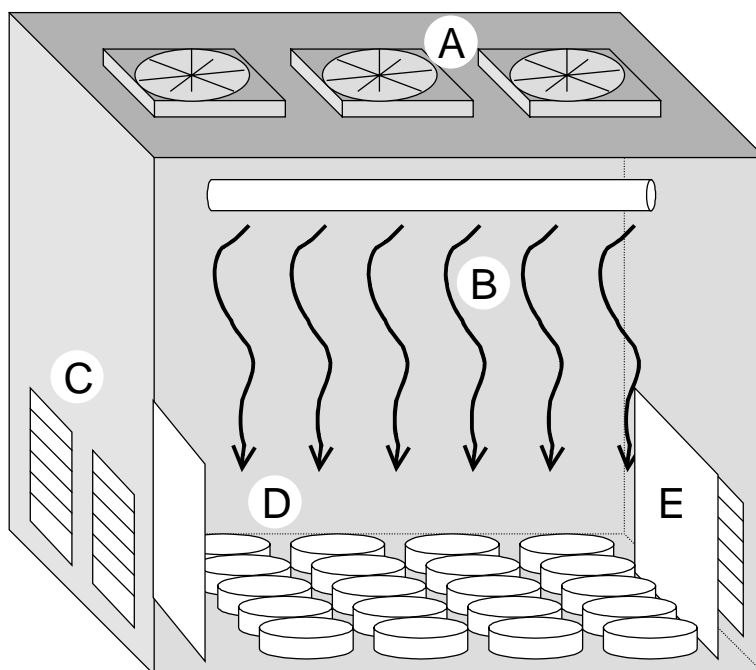

Figure 2. Pulsed-UV collimated beam apparatus

A: Fans installed on the roof of the light box to exhaust heat of convection
B: Heat of radiation
C: Vents installed at the bottom sides of the light box
D: Glass-covered petri dish samples placed at the center of the box
E: Air-vent baffles placed along the sides of the samples to inhibit evaporation

Figure 3. Light incubation chamber for photoreactivation experiments
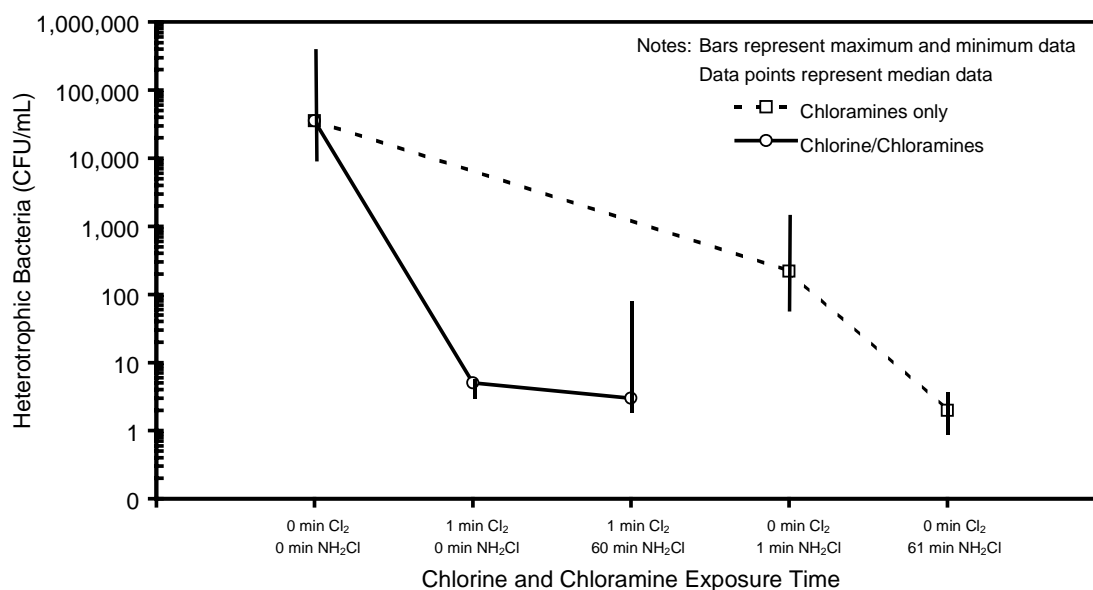
Figure 4. Disinfection of heterotrophic bacteria with chlorine and chloramines
(median, minimum, and maximum values from three replicate tests)
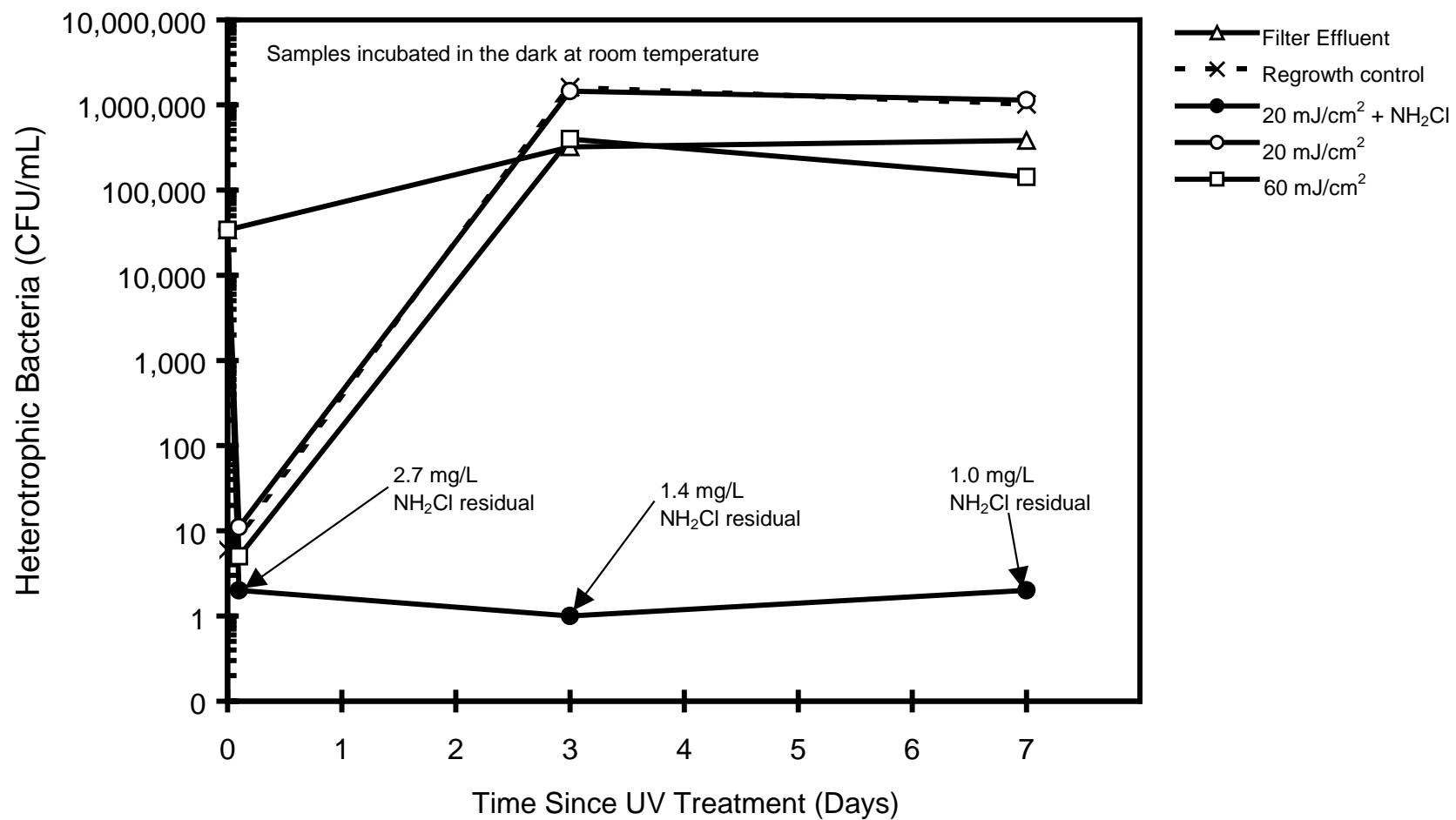
Figure 5. Effect of incubation in a simulated distribution system on heterotrophic bacteria after exposure to medium-pressure UV light
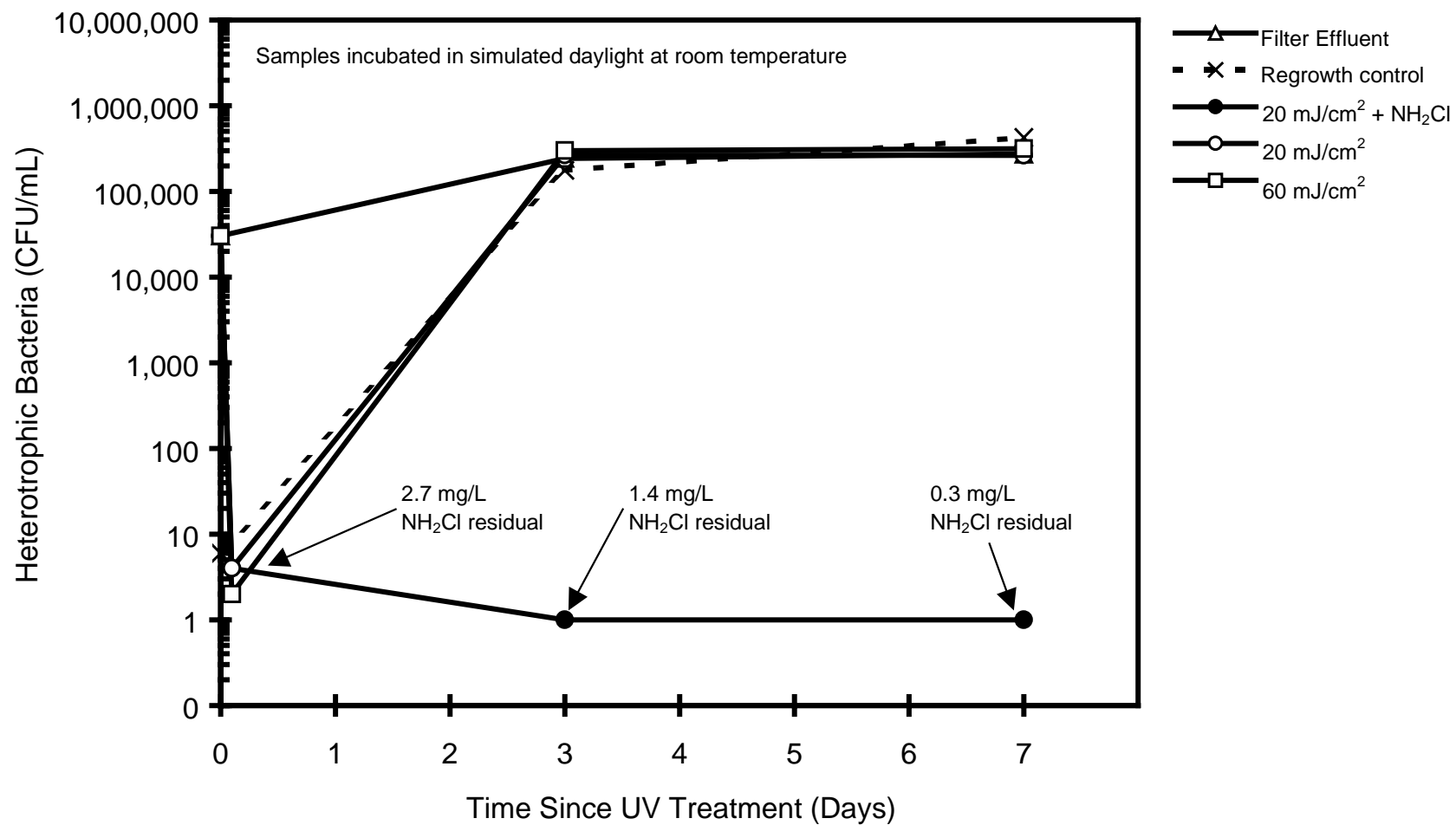
Figure 6. Effect of incubation in a simulated daylight environment on heterotrophic bacteria after exposure to medium-pressure UV light
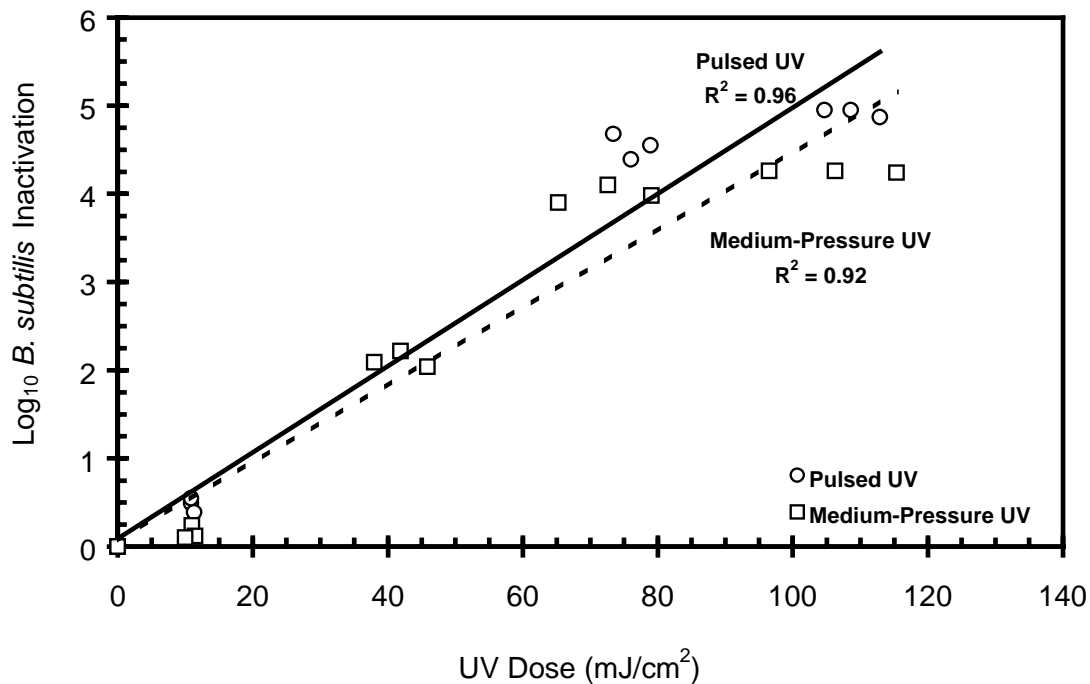
Figure 7. Effect of medium-pressure and pulsed-UV light on
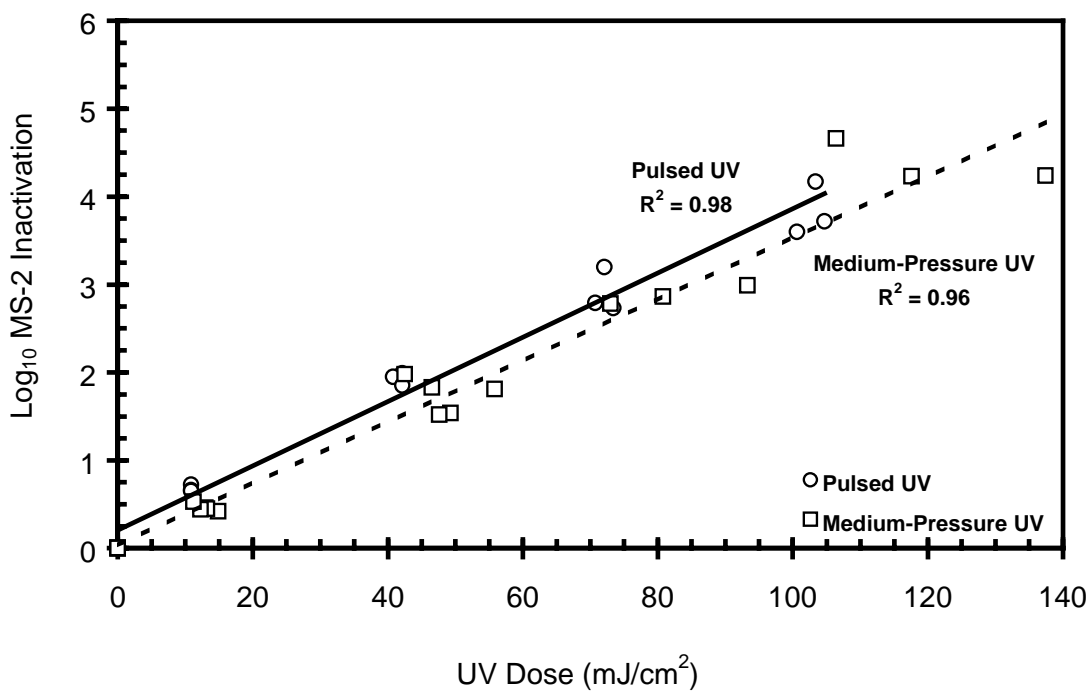
Bacillus subtilis spores

Figure 8. Effect of medium-pressure and pulsed-UV light on MS-2 coliphage

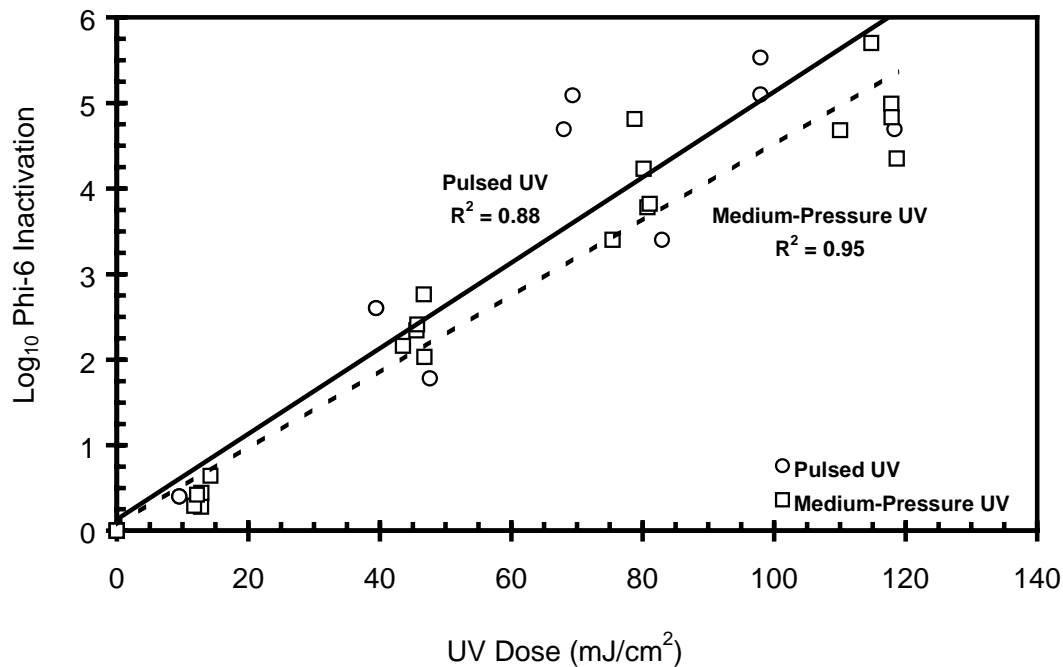(single-stranded RNA virus)

Figure 9. Effect of medium-pressure and pulsed-UV light on phi-6 bacteriophage
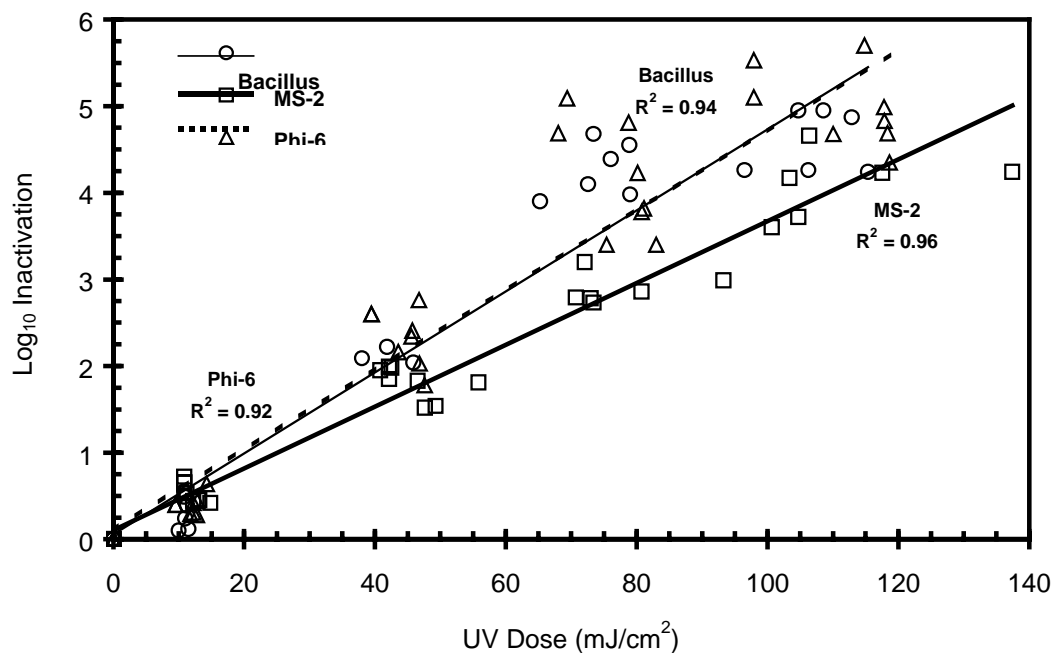(double-stranded RNA virus)

Figure 10. Effect of polychromatic UV light from both medium-pressure and pulsed UV
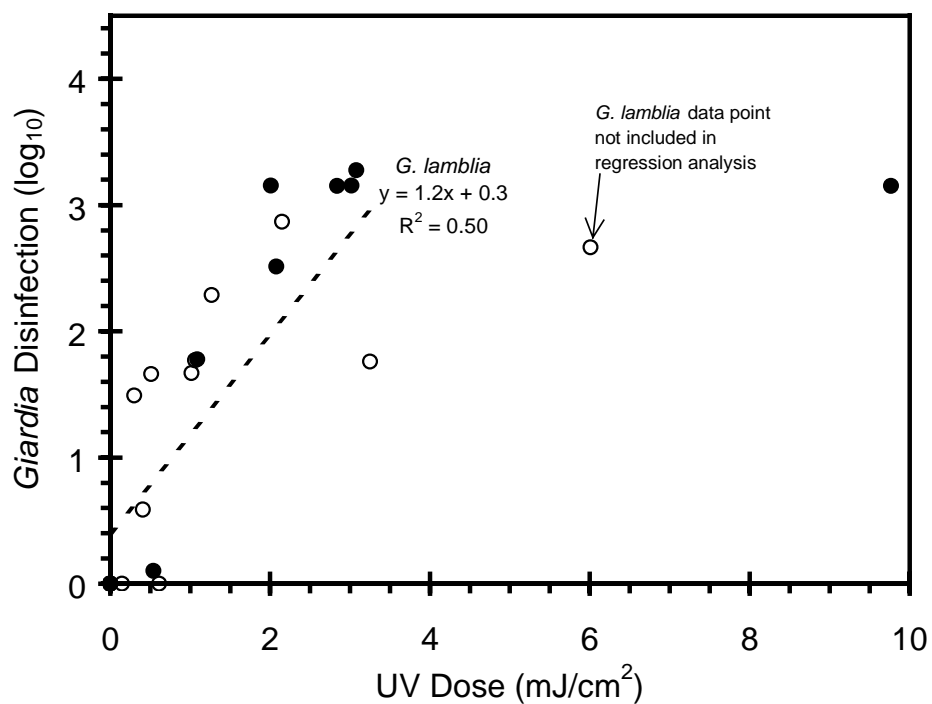on *B. subtilis*, MS-2 coliphage, and phi-6 bacteriophage

Figure 11. UV dose-cyst response for *G. lamblia*

(filled data indicate data beyond detection limit of the infectivity assay)
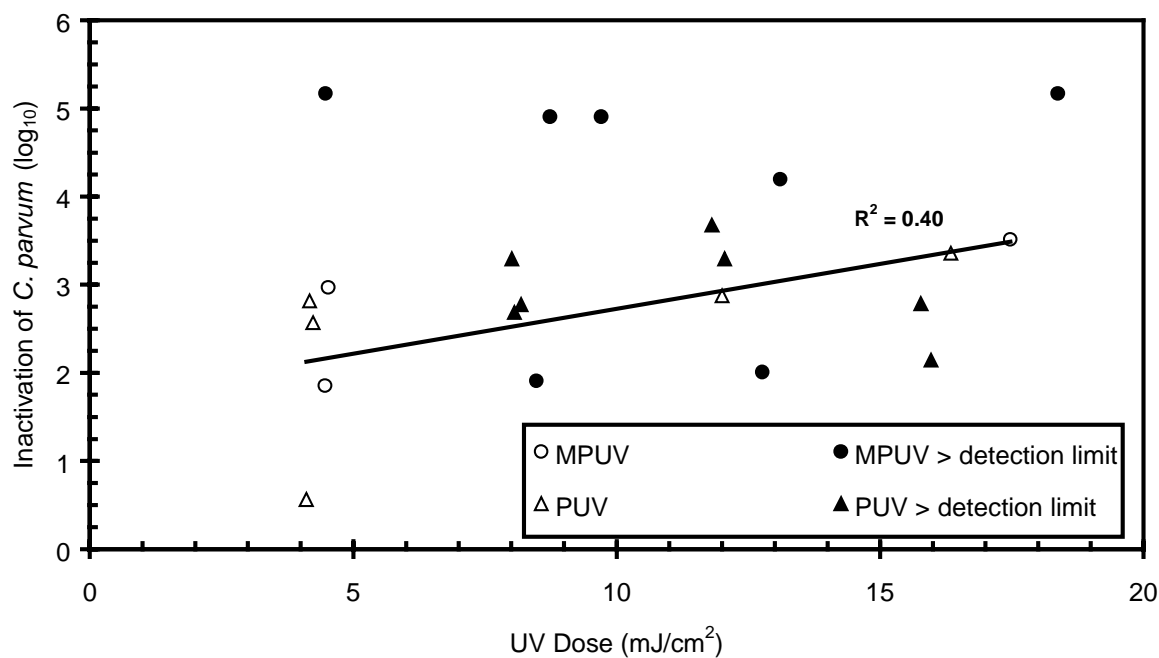

Figure 12. Reduction in *C. parvum* oocyst infectivity pre-incubation

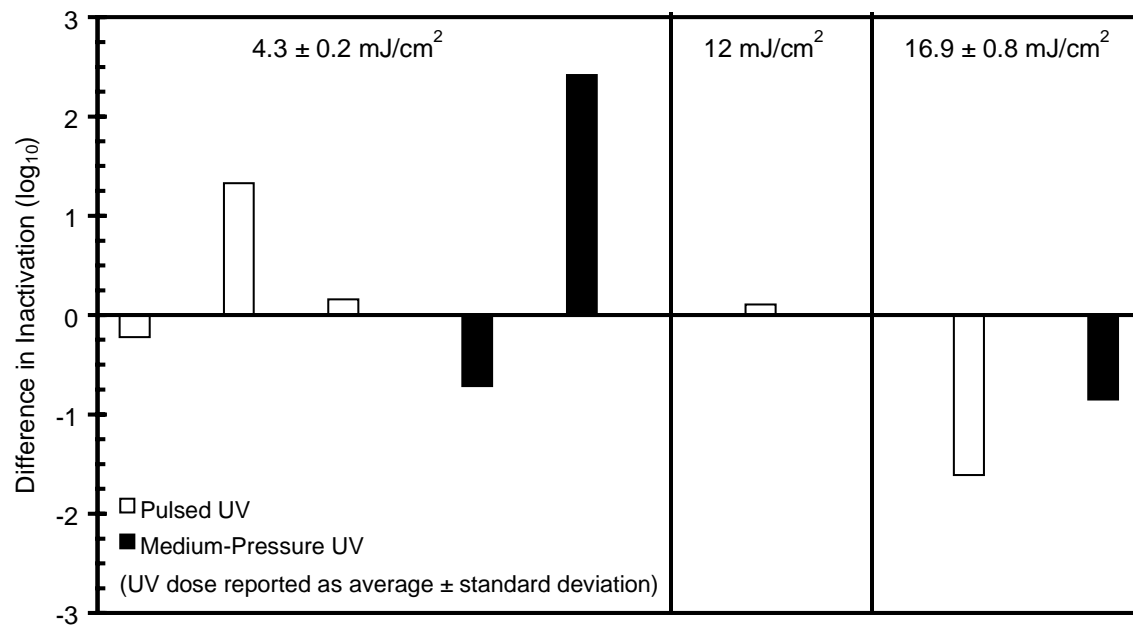(filled data indicate data beyond detection limit of the infectivity assay)

Figure 13. Difference in *C. parvum* oocyst infectivity after 7-day incubation

(decrease in infectivity indicates possible repair of oocysts)

GLOSSARY

ATCC   American Type Culture Collection

B. subtilis   Bacillus subtilis

bp   base pair

$CaCO_3$ calcium carbonate

Commission   California Energy Commission

cm   centimeter

R^2 coefficient of determination

CFU/mL   colony forming units per milliliter

C. parvum   *Cryptosporidium parvum*

d   day

^{o}C   degrees Celcius

^{o}F   degrees Farenheit

DNA   deoxyribonucleic acid

DBP   disinfection byproduct

E. coli   *Escherichia coliform*

EDTA   ethylenedinitrilo tetraacetic acid

gpm   gallons per minute

GAPDH   glyceraldehyde-3-phosphate dehydrogenase

G. lamblia   *Giardia lamblia*

G. muris   *Giardia muris*

g   gravitational pull

hr   hour

HAA	haloacetic acid
hsp	heat-shock protein
HCT-8	human illeocecal carcinoma cells
I.D.	identification
in	inch
ID_{50}	dose that would infect 50 percent of the population inoculated
kWh	killowatt-hour
log_{10}	logarithmic (base 10)
R2A	low-nutrient agar for heterotrophic bacteria assay
Metropolitan	Metropolitan Water District of Southern California
µg/L	micrograms per liter
µL	microliter
µm	micrometer
mg/L	milligrams per liter
mg/mL	milligrams per milliliter
mJ/cm^2	millijoules per square centimeter
mL	milliliter
mm	millimeter
MGD	million gallons per day
mW/cm^2	milliwatts per square centimeter
min	minute
MPN	most probable number
pH	the negative log_{10} concentration of the activity of hydrogen ions

NBY   nutrient broth yeast extract

OHSU   Oregon Health Sciences University

Tween 20   polyoxyethylenesorbitan monolaurate

PFU/mL   plaque forming units per milliliter

PVC   polyvinyl chloride

phi-6   pseudomonas syringae pathovar phaseolicola bacteriophage phi-6

PIER   Public Interest Energy Research

RD&D   research, development, and demonstration

RT-PCR   reverse-transcriptase, polymerase chain reaction

RNA   ribonucleic acid

sec   second

THM   trihalomethane

USEPA   United States Environmental Protection Agency

UV   ultraviolet

W   watt

WB   WB strain of *G. lamblia*